Introduction

During metastasis, we see epithelial tumor cells invading the surrounding extracellular matrix (ECM), disseminating into blood circulation and establishing secondary tumors in distant sites.

To do so, the stationnary epithelial cells lose their epithelial characteristics (Including adherent junctions and basal apical polarity), and acquire a mesenchymal morphology and the ability to migrate and invade other tissues, a process that is known as epithelial-mesenchymal transition (EMT).
At a biochemical level, cells switch off the expression of epithelial markers (such as E-cadherin and catenins) and turn on mesenchymal markers (such as vimentin and fibronectin). Previous studies (using cell culture and tumor xenograft models) show that activation of EMT promotes carcinoma cells to dissociate from each other and metastasize to distant organs.

EMT is controled by a network of transcription factors including Twist1, Snail1/2, Zeb1/2 and FOXC2. Their previous study shows that Twist1 is a potent inducer of EMT and ECM degradation. In mouse and human breast cancer tumor xenograft, Twist1 can promote tumor metastasis, its expression in primary tumors has also been related to desease agressiveness and poor survival in many types of human cancer.
Recent studies using atochthonous mouse tumor models observed the occurrence of EMT in primary carcinoma but how EMT spatiotemporally regulates metastasis has not been investigated in these models.

However, the involvement of EMT *in vivo* is controversial since, in human carcinoma, although primary tumors show many morphological and molecular features of EMT in subpopulations of invasive cells, metastases present an epithelial morphology. Authors suggest that this discrepency could be due to the misinterpretation of the EMT as a permanent, non reversible process. A reversible EMT model has been proposed in 2002 to explain the epithelial identity of metastatic cells but it has not been attested *in vivo*.

The carcinogenesis mouse skin model has been shown to recapitulate the multistep process of human carcinoma progression (Including initiation, growth, invasion and metastasis) and shows strong similarities human carcinoma. Like human squamous cell carcinoma, it develops metastasis with an epithelial morphology in lymph nodes and lungs, making it a suitable model to study the involvement of EMT *in vivo*. In this article, the authors investigate the importance of a dynamic EMT pocess in metastasis.

Figure 2 :

Diapo 1 :

To understand how irreversible versus reversible induction of Twist1 impacts metastasis, the authors examined individual mice for distant metastases by macroscopic and histological analysis.

(A) and (B): They sacrificed mice from 5 to 8 weeks after doxycycline induction to look for metastasis. Consistant with published data, 27-33% of control SCC (Squamous Cell Carcinoma) mice developed distant metastases in the lymph nodes and/or the lung.
Strikingly, 12 out of 14 K5-Twist1 mice (86%) receiving topical doxycycline developed distant metastases. In contrast, only 3 out of 13 K5-Twist1 mice (23%) receiving oral doxycycline developed distant metastases.

We see that mice receiveing topical injection of doxycycline (mimicking reversible induction of Twist1) develop significantly more frequently metastases and more metastatic lesions that mice receiving oral doxycycline and control.

Diapo 2 :

(C): They next examined the expression of Keratin 5 and Twist1 in the primary tumors and metastatic nodules. They found that all skin tumor cells express Keratin 5 both with and without Twist1 induction, suggesting that Keratin 5 can be used to specifically mark skin tumor cells in this model.

Reversible induction of Twist1 promotes carcinoma metastasis

Figure 4 :

Diapo 1 :

To successfully metastasize, carcinoma cells need to complete distinct steps, including invasion, intravasation, extravasation, and growth at distant sites.

(A): To investigate how activation of EMT impacts tumor cell intravasation into the blood circulation, whey isolated circulating tumor cells (CTCs) from peripheral blood of K5-Twist1 and control mice. CTCs are defined as cells that are CD45- and pan-cytokeratin (CK)+ and present irregular nuclear shape.

(B) The percentage of CTCs in blood is significantly higher in K5-Twist1 mice treated with doxycycline than in control mice and K5-Twist1 mice prior to doxycycline treatment.

Diapo 2 :

(C) Upon Twist1 induction, these CTCs were positive for Twist1 and mesenchymal marker vimentin, but negative for epithelial markers E-cadherin and β-catenin, indicating an EMT phenotype in the CTCs.
Consistant with studies showing that CTCS from human cancer patient present many features of EMT.

The data show that both reversible and irreversible activation of EMT are equally effective in promoting tumor cell intravasation.

Figure 6 :

Diapo 1 :

Since reversible and irreversible activation of EMT can both effectively promote local invasion, intravasation, and extravasation, the ability to grow in distant organs is likely to be the critical step regulated by the reversion of EMT.
Cell proliferation is essential for establishing macrometastases and EMT-inducing factors are shown to reduce cell proliferation.

(A) Twist1 on tumor cell proliferation. Indeed, individual primary tumor cells expressing Twist1 showed very low to non-detectable expression of the proliferation marker Ki67

(B et C) More thant that, tumor cell proliferation appeared to be negatively correlated with Twist1 expression

Diapo 2 :

(D) Early metastatic colonies showed strong positive Ki67 expression and low Twist1 expression under “reversible” EMT condition, while “irreversible” EMT resulted in colonies with high Twist1 expression and low Ki67

This demonstrates that reversion of EMT promotes proliferation and establishment of early metastatic colonies in distant sites